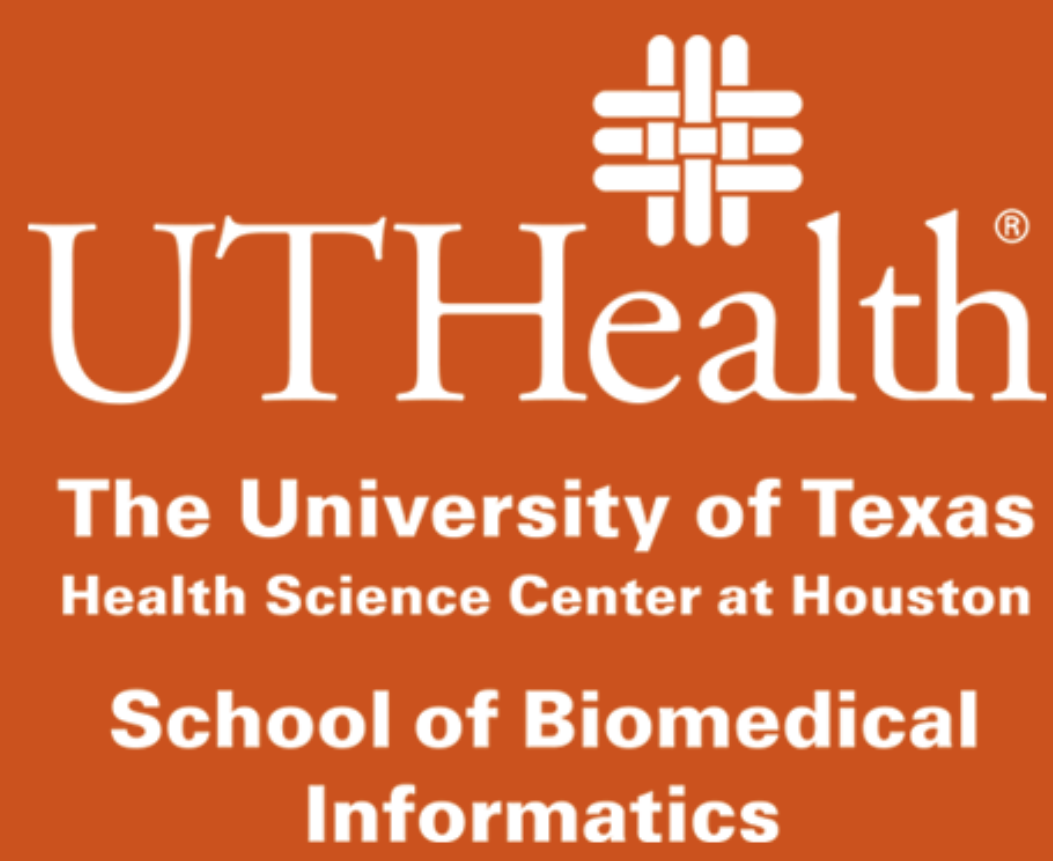


Epigenetic regulation of bone stem cell lineage under cytokine treatment

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Introduction

The process of bone tissue regeneration involves a cascade of genetic and epigenetic factors that drive both the direction and magnitude of bone stem cell lineage. In our previous study, we revealed that sequential delivery of two growth factors, BMP2 and IGF1, in a particular temporal order, rather than simultaneously, promotes the optimal calcium deposition. We further figured out the underlying molecular mechanisms that well explain the phenotypic observations, by integrating experimental data, mathematical model and bioinformatics analysis. Specifically, the two growth factors trigger different signaling pathways which orchestrate and converge to guide the osteoblastic lineage commitment in a sequential manner. We also determined three critical transcription factors (TFs) that are critical for this complex biological process, including Runx2, Osterix and β -catenin. However, in our previous study and studies conducted by other groups, the potential regulatory factors were only investigated at the gene/protein expression level, while the epigenetic regulation profiles including histone modification (HM) and TF binding patterns were poorly explored.

To address this gap, we extended our preliminary study by further checking TF binding and HM patterns over time under cytokine treatment. By a comprehensive analysis on ChIP-seq data of two TFs (CTCF and EP300) and 10 HM markers in human osteoblast from ENCODE, we found H3K4me2, H3K4me3 and H3K79me2 are highly enriched in the gene body and/or TSS region of all three critical TFs. Integrative analysis of DNase-seq data of bone marrow derived stem cells (BMSC) and osteoblast further revealed the dynamic change of chromatin accessibility along the BMSC lineage process.

In our ongoing efforts, we are focusing on the most important TFs Runx2 and Osterix and check their dynamic genome-wide occupancy, with or without cytokine treatment. To achieve this, we perform ChIP-seq assay with MC3T3-E1 preosteoblasts on day 0, 7, 14 and 28 under each condition. We will characterize both the binding sites and binding intensity of each TF under various treatment regimens in a time-dependent sense. Our study is expected to address the previously missing chain pertaining to the epigenetic regulation of bone stem cell lineage process under cytokine treatment at a high temporal resolution.

Preliminary Results

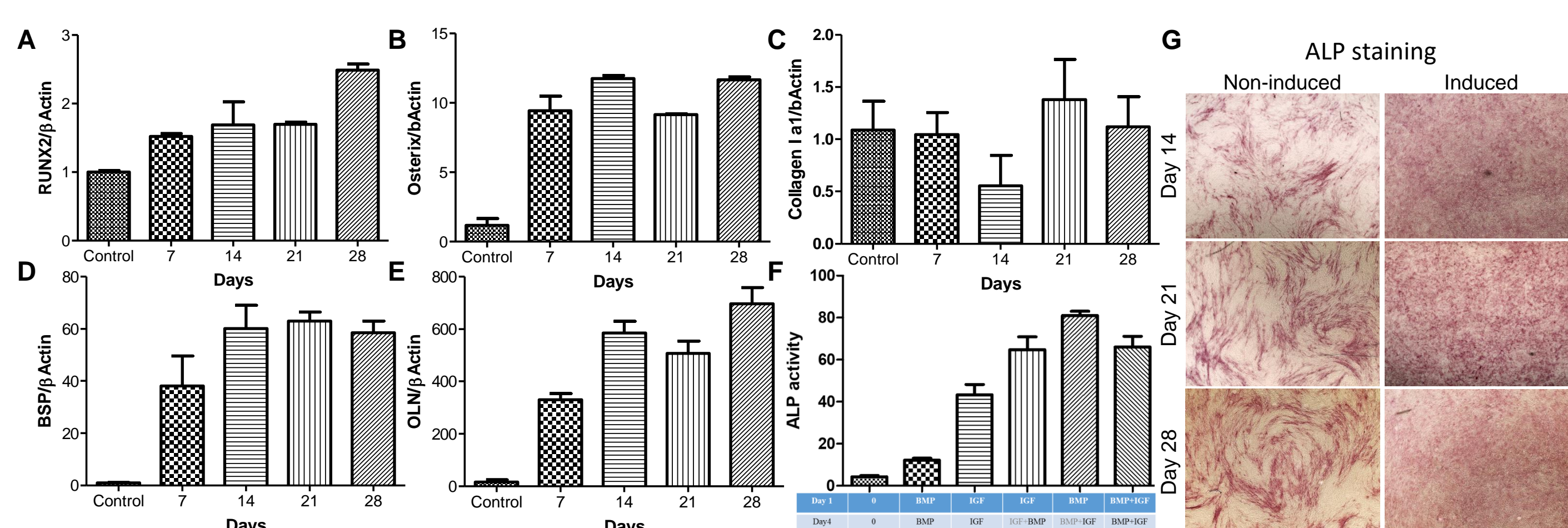


Fig. 1. (A-E) Dynamic change of bone lineage related biomarkers on day 0 (“Control”), 7, 14, 21 and 28, measured by quantitative real-time PCR (qPCR), with β -actin as loading control. (F) The osteoblastic differentiation biomarker ALP were measured under different treatment scenarios. Mouse preosteoblast cells MC3T3-E1 were treated by two growth factors BMP2 and IGF1 in a sequential manner as indicated in the table. (G) ALP staining were conducted under two conditions: naturally and medium induced. BSP: bone sialoprotein; OLN: osteocalcin; ALP: alkaline phosphatase.

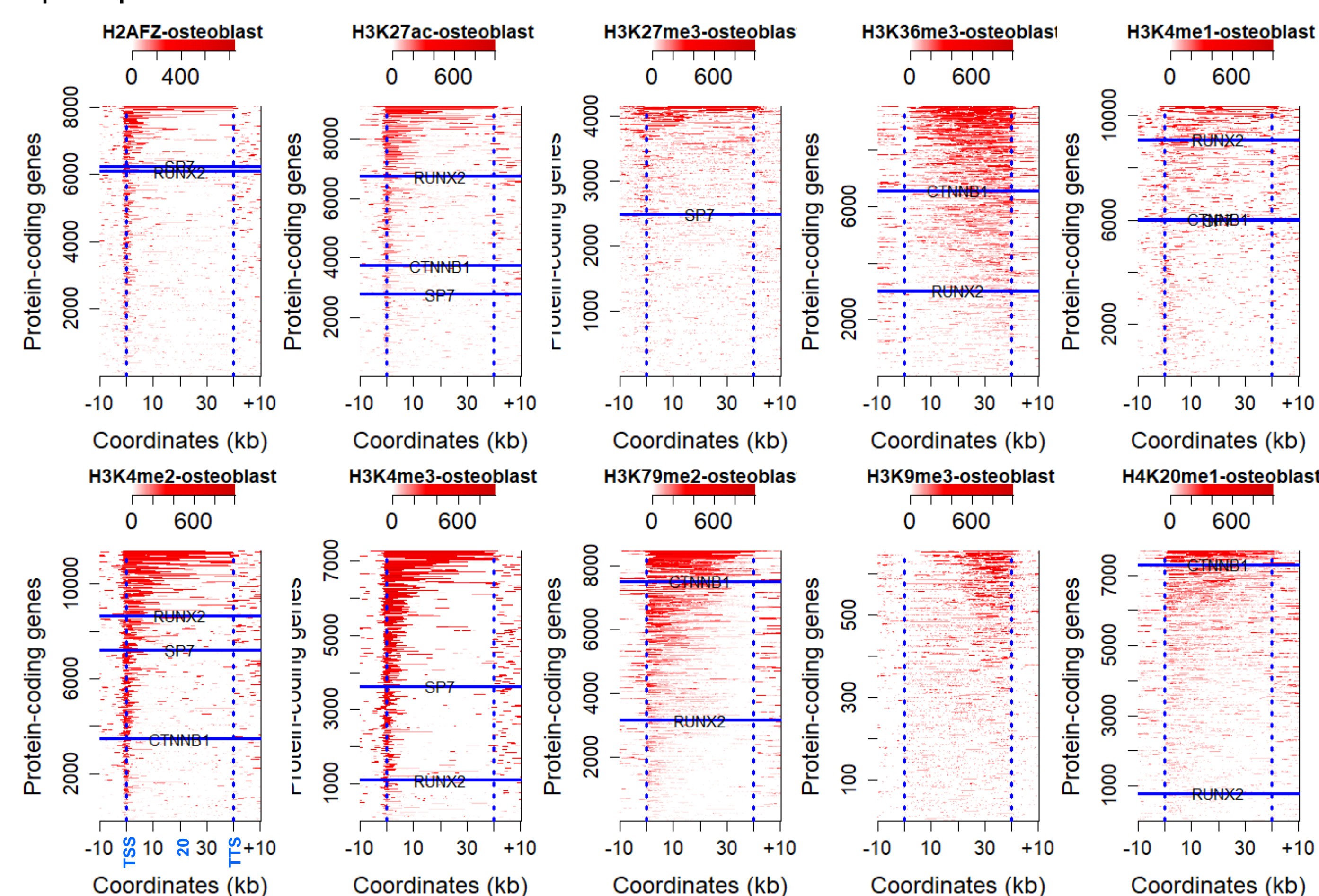


Fig. 2. Heatmap showing density of indicated histone modification (HM) marks in osteoblast cells. ChIP-seq data were retrieved from ENCODE database. Columns represent the region from -10 kb w.r.t TSS to +10 kb w.r.t TTS. This region for each gene was divided into 300 bins as follows: the gene body was divided into 200 bins and each flanking 10 kb region was divided into 50 bins. In this way, all genes were scaled to be 40 kb long. For each HM marker, genes were ranked by number of bins with detectable epigenetic signal. Only genes with ≥ 20 detectable bins are shown. The ranking position of RUNX2, SP7 (encoding Osterix) and CTNNB1 (encoding β -catenin) is marked blue in each sub-figure.

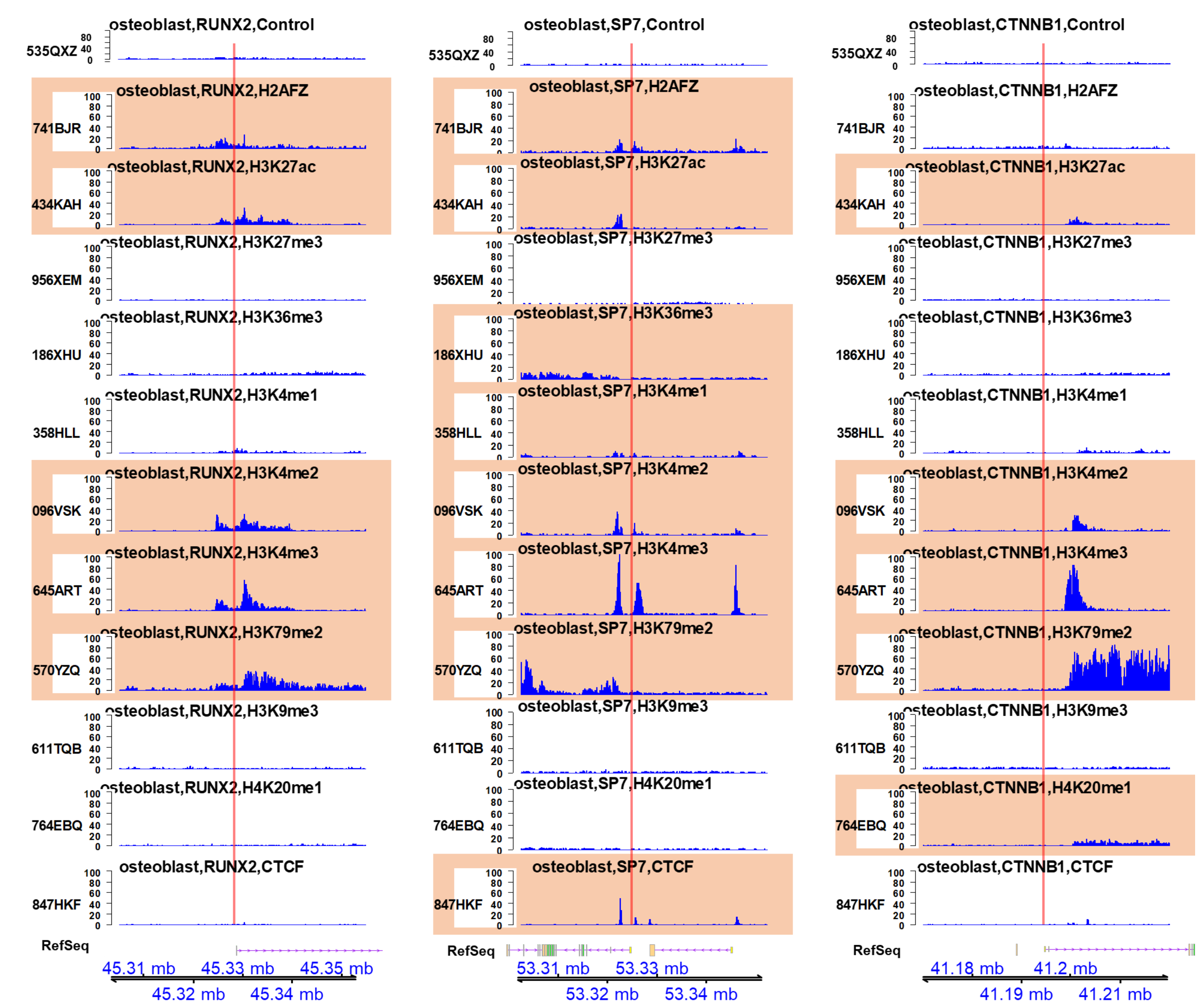


Fig. 3. Enrichment profiles of epigenetic factors surrounding TSS of RUNX2, SP7 and CTNNB1. ChIP-seq data for osteoblast cells from ENCODE for 10 histone modification (HM) markers and CTCF, as well as Control were indicated in each sub-figure. Shown region is TSS \pm 50kb of each gene. H3K4me2, H3K4me3 and H3K79me2 are significantly enriched in the region of all three TFs. H2AFZ and H3K27ac were enriched in RUNX2 and SP7 region.

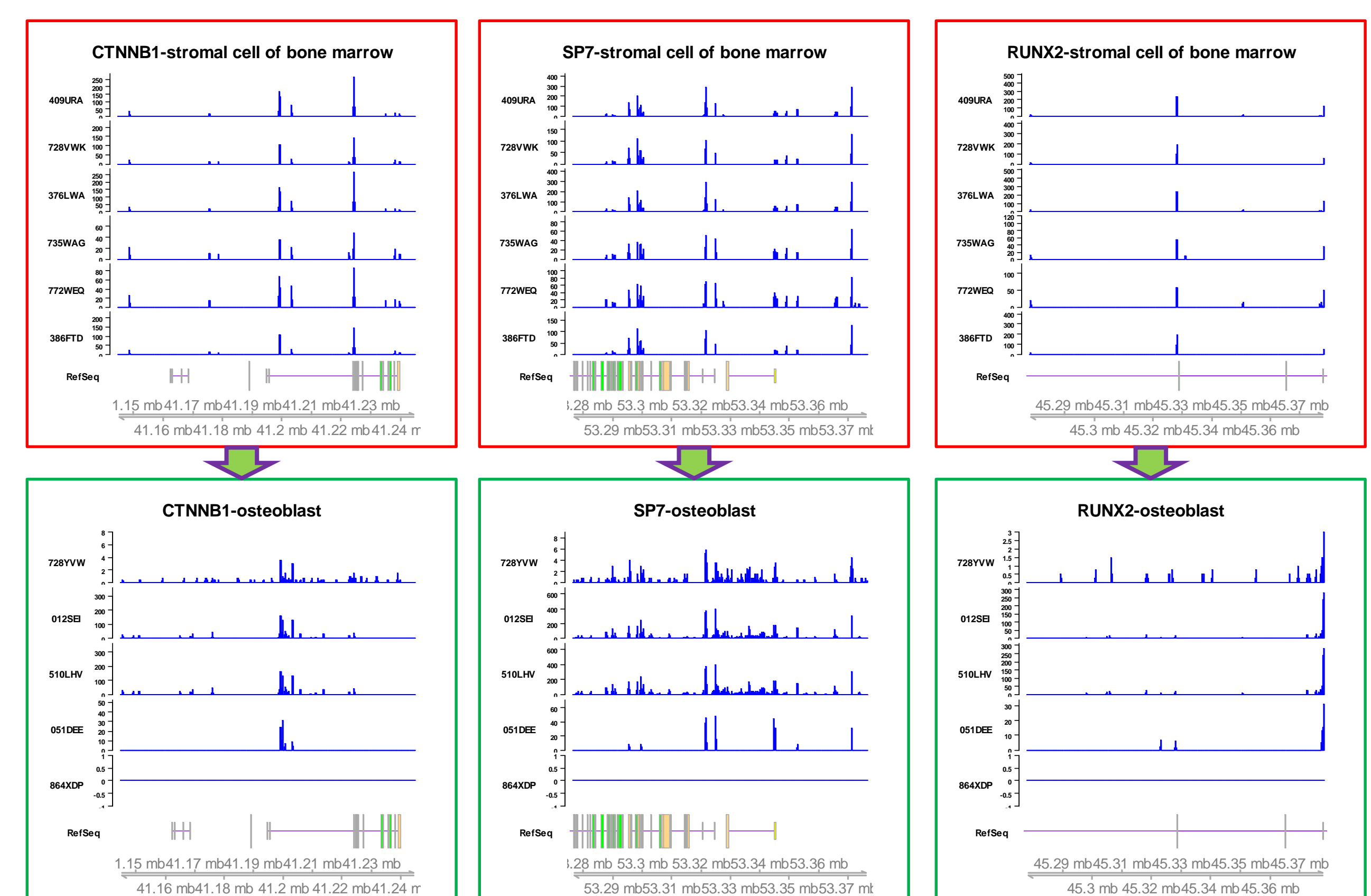


Fig. 4. Chromatin accessibility profiles in the region of RUNX2, SP7 and CTNNB1 along osteoblastic lineage process detected by DNase-seq from ENCODE. Shown region is TSS \pm 50kb of each gene. Generally wide DNase enrichment region was observed during the lineage commitment from stromal cell of bone marrow to osteoblast, indicating increased DNA accessibility in the TSS region of three TFs.

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